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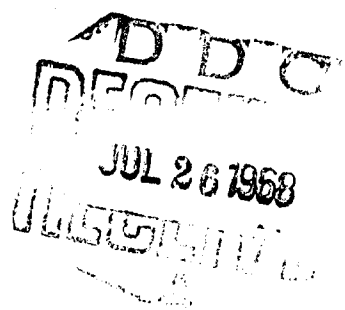

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

THE FUNCTION OF THE COMPLEMENT IN EXPERIMENTAL ANTHRAX INFECTIONS

- Italy -

/Following is the translation of an article by Dr. Aldo Ceccarelli in the Italian-language monthly Zooprofilassi, Rivista Mensile Di Scienza e Tecnica Veterinaria (Zoological Prophylaxis, Monthly Review of Science and Technology) Vol 2, No 12, Rome, December 1947, pp 341-346./

Original Article

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Alexin or complement is present in the blood of animals, including cold-blooded animals, but not in birds, although not all species of doves are always absolutely free from it. However, there are notable differences in the complement content according to the animal species and the individual. The animal whose serum is normally rich in complementary capacity is the guinea pig.

When speaking generally about alexin, its characteristics, and its manner of action, its composition and origin, we are still rather in the dark. Based on the latest scientific findings we are able to divide the complement into five parts of which two would be thermostabile and three thermostabile. These partial units are inactive whenever separated, but in conjunction with each other they can exercise a complementary force. These findings let us assume that the complement is not a chemically definable element, but that its

capacity results from a special condition of the colloidal serum-complex which is easy to change by means of a variety of chemical and physical agents.

This is why it seems to be more reasonable to speak about a complementary action, rather than about the complement itself. If we admit having only little knowledge with respect to the characteristics and the composition of the complement, we know even less about the interaction between complementary force on the one hand and susceptibility to germs and bacterial toxins on the other.

We know that there is no parallelism between natural immunity and normal bactericidal action, but on the other hand there is no doubt that in an acquired antibacterial immunity, bacteriolysins play a predominant part, the existing protective action against germ infection being due only to these agents. Such an antibody is formed by two substances: amboceptor and complement, and naturally there can be a lack of bacteriolytic capacity in the serum if one of these substances is missing or is present in excess, so that a definite quantitative relationship between these substances is absolutely necessary.

There are many factors which under normal conditions might reduce the complement and consequently also the germicidal action. The simplest way to develop germs in animals who by their nature are refractory in order to make them susceptible to disease (normally achieved by means of various devices, such as underfeeding, avitaminosis, fatigue, etc.) may perhaps be an adequate reduction of alexin. Lusena showed that the rabbit, being naturally almost non-susceptible to typhoid, dies in a short time when the complement is inactivated judiciously and De Antoni observed that even minimum doses of germs are sufficient to kill the rabbit as soon as its normal defensive capacities are paralyzed by inactivation of complement. However, the question about the importance of complement with respect to many other infectious diseases still remains unsolved and has so far been merely evaluated in the light of a number of experimental observations.

Weber found already in 1935 that the organic resistance of a subject seems to depend upon the complement content of its blood.

More recently Canezza tested the action of fresh bovine serum which contained complement; he applied it in serotherapy against cattle plague and obtained best results with

eight sick calves of which seven were cured. Such a successful outcome of an experiment should be taken as real confirmation of the theory of the importance of complement in the resistance of an animal against infection. On the other hand it is already well-known in practice that the action of anti-erysipelas serum is increased by a simultaneous injection of absolutely fresh horse serum (Stazzi).

The importance of complement as related to immunity against carbunculosis has also been widely studied in the field to hematic carbunculosis. In the blood of animals afflicted with or cured of such diseases agglutinins, precipitins, and a sensibilizer have been found although the importance of these factors as related to the creation of immunity against carbunculosis has not yet been established despite all the long research work done to this end. According to some authors, the present importance of such antibodies is very uncertain because they observed a lack of connection between immunity and lytic action of the humors. For example, Petragnani found that the carbuncle germ lives well in the blood of the dove at a temperature of 37° C and that also the blood of immunized pigeons has no lytic effect upon the germ itself. Carpano found that chicken blood has no germicidal quality and Mazza carried out tests to study the bactericidal power and components of the blood of various species of animals, eventually coming to the conclusion that in susceptive subjects as well as in naturally immune ones the actual seat of anti-carbunculosis immunity is presumably located neither in the blood nor in one of its components.

Castellana, in turn, could probably have established that whole pigeon blood possesses outstanding anthracidal qualities, as Sani had already observed.

These contradictory findings show us that we are still very much in the dark about the characteristics and the root of immunity against carbunculosis. This was the reason why we desired to complete research designed to emphasize the more or less high importance of complement in producing immunity against carbunculosis in susceptible animals. We found it preferable to proceed with living subjects, giving us a chance to get as close as possible to natural conditions.

We obtained the complement inactivation of rabbits by means of inoculation with "Neojacol"; according to Felke this product possesses anticoagulant action because it is supposed to bind the fibrinogen of the blood, while Lusena causes us to assume the anti-coagulant quality to be due in

the first place to an anti-prothrombinic action. Lucena himself revealed the anti-complementary action of "Neojacol" because when added in vitro to rabbit blood at the rate of 2 mg per cc it makes the blood indefinitely non-coagulable, free from germicidal ability, and from complementary action. An injection of the same substance into living animals at the rate of 0.20 g per kg of body weight will lead to the non-coagulability and absence of complement for at least 24 hours. Under observation of the aforementioned doses rabbits generally tolerate the drug without particular trouble, except for an occasional slight reduction in weight. We might sometimes find subjects in which the inactivation of complement lasts not quite as long as in others and in that case it is advisable to test the rabbits before inoculation for such an eventual lack of complementary capacity. In view of the fact that such phenomena as non-coagulability and lack of complement are equivalent among themselves it is sufficient to establish the non-coagulability of the blood in order to find the simultaneous lack of complement. The purpose of our work was:

1. To find, by means of tests, whether it is possible to infect rabbits with carbunculosis after having immunized them previously with anti-carbunculosis serum and then inactivated their complement.

2. To find whether and to what degree a carbuncular septicemia can be produced in rabbits previously vaccinated and later with their complement inactivated, if we inoculate them with a virulent substance.

...

The tests were carried out on a lot of 22 rabbits, divided into one group of 14 and another of eight.

The animals were in excellent conditions of health and nourishment, weighting from 1.100 kg to 1.800 kg approximately, many of them belonging to the same litter. The tests took place from 16 December 1946 to 20 April 1947.

First Test Series

We were working with 14 rabbits, marking the first nine of them progressively from 1 through 9 and the other five with numbers 1c, 2c, 3c, 4c and 5c. On 16 December 1946 each rabbit, except Nos 6, 7, 8 and 9 received an endoperitoneal injection of 10 cc of anti-carbuncular serum of the

1 S.K. type. On 18 December 1946 rabbits Nos 6 and 7 were inoculated intravenously in the left auricle with 5 cc of sterile distilled water containing 20 cc of "Neojacol" per kg of body weight or more precisely speaking: Rabbit No 6, weighing 1.100 kg, got 22 cg of the drug while the other one, No 7, weighing 1.200 kg, was given 24 cg of "Neojacol".

Rabbits Nos 8 and 9 were inoculated subcutaneously with 1 cc of a 48 hour broth culture of bacillus anthracis. Rabbits Nos. 1, 2, 3, 4, and 5 were inoculated with 5 cc of sterile distilled water, containing a solution of 20 cg of "Neojacol" per kg of body weight. This was done 48 hours after their inoculation with anti-carbunculosis serum.

Right after the injection of the drug the same rabbits were inoculated subcutaneously each with 1 cc of a 48 hour broth culture of bacillus anthracis extracted from calf spleen whose pathogenic capacity had been previously tested upon guinea pigs and rabbits. The same day rabbits Nos. 1c, 2c, 3c, 4c, and 5c were inoculated subcutaneously with 1 cc of carbuncular broth culture.

Rabbits Nos. 6 and 7, inoculated with "Neojacol" only, served as control animals. Rabbits Nos. 1, 2, 3, 4, and 5, immunized with anti-carbuncular serum, had the complement inactivated with "Neojacol" and then inoculated with anthrax. Rabbits Nos. 1c, 2c, 3c, 4c. and 5c, immunized with anti-carbuncular serum, were inoculated with anthrax only.

We observed the behavior of all the individual animals after their inoculation. Rabbits Nos. 6 and 7, treated only with "Neojacol", did not show any disorder whatsoever and after killing them two months later nothing noteworthy was revealed in the necroscopy. A test taken on the same day of the inoculation to try the complementary capacity (titration for complement with a hemolytic system) gave evidence of a considerable reduction in complement, compared to what it was before the treatment and furthermore, a lack of coagulability of the blood.

Rabbits Nos. 1, 2, 3, 4 and 5, which had been immunized, had had their complement inactivated and later were infected, all died in a period of time ranging from 48 to 72 hours. Anatomicopathological, microscopic and cultural examinations made on this group of animals gave the following results:

Rabbit No. 1 died within 50 hours with typical symptoms of carbunculosis (secretion of hemorrhagic gelatinous

serum at the point of inoculation, splenomegaly, etc.) The microscopic and cultural report was positive.

Rabbit No. 2 died after 62 hours; the point of inoculation showed a gelatinous serous exudate with capsulated germs; the internal organs were swollen and congested, the spleen almost normal, the blood not coagulated. Liver, spleen and blood tests of the heart did not reveal any carbunculosis germs. From a sample of heart blood we prepared a simple culture on agar and broth which after 24 hours gave a positive result as to the existence of bacillus anthracis.

This fact allowed us to verify the former statements of other authors that animals treated with anti-carbunculosis serum can be infected immediately and die without evidence of the germ in the organs where it is normally found in large quantities.

Of rabbits Nos 3 and 4 one died within 52 hours and the other within 70 hours, the anatomicopathological, microscopic and cultural report being positive for carbunculosis.

Rabbit No 5 died within 40 hours, and its report was similar to the one of rabbit No 2, with the absence of bacillary elements in the microscopic test and development of germs in sterile conditions, according to the culture tests.

Rabbits Nos 1c, 2c, 3c, 4c and 5c, immunized with serum and infected with anthrax stayed alive for a considerable amount of time.

Rabbit No 1c died 27 December, i.e. 9 days after its infection.

The anatomicopathological, microscopic and culture report was absolutely negative with respect to carbunculosis. In view of numerous oocytes found in the intestine, resulting from catarrhal enteritis, it was thought that the death of this young animal was due to coccidiosis.

Rabbit No 2c died 2 January 1947, i.e. 15 days after its carbuncular inoculation. The anatomicopathological report was negative, while the culture test showed the injection germs. It is assumed that the anti-carbunculosis serum has been protecting the animal during the above period, whereafter the rabbit, becoming again susceptible to carbunculosis, died of an infection contracted from its environment.

Rabbits Nos 3c, 4c and 5c stayed alive without any disorder whatsoever. Rabbits Nos 8 and 9, having inoculated with anthrax only, died of typical carbuncular septicemia within 34 to 46 hours afterward.

Observations Concerning the First Test Series

The rabbits passively immunized against carbunculosis and later with their complement inactivated with "Neojacol", died of septicemia in 100% of the cases, whereas the control subjects treated with seroprophylaxis, later inoculated with anthrax, failed to react at all to the pathogenic effect of the germ in 80% of the cases. 20% of the animals reacted only slowly, when the protective effect of the serum had already disappeared.

Second Test Series

For this series we used eight rabbits. We did not find it necessary to repeat the control proceedings with shots of "Neojacol" only, or with anthrax only, as this had been done already with subjects Nos 6 and 7, and 8 and 9 respectively, during the first test series.

Out of the eight 1.200 kg medium-weight rabbits the first six were vaccinated subcutaneously with "Carbozoo" anti-carbuncular vaccine on 25 March 1947. On 8 April 1947, 13 days after the vaccination, rabbits Nos 1, 2, 3, 4, 5 and 6 were inoculated with ½ cc of a 48 hour broth culture of anthrax bacteria.

The animals only showed a slight swelling at the point of the inoculation, without any other disorder.

On 13 May 1947 rabbits Nos. 1, 2, 3, and 4 had the complement inactivated and immediately inoculated subcutaneously with 0.5 cc of a 48 hour broth culture of bacillus anthracis, containing germs and spores. Rabbits Nos 5 and 6, which also had been previously vaccinated and checked, were not reinoculated with 0.5 cc of broth culture.

As control specimens with anthrax only, we used the rabbits of our first series of tests and at the same time they were used as a control group for "Neojacol".

Within two to three days rabbits Nos. 1, 2, 3 and 4 and the two control animals inoculated with anthrax only

died, while rabbits Nos. 5 and 6 stayed alive and remained in perfect conditions of health. The anatomicopathological, microscopic and cultural inspections showed presence of the injected germ.

Observations About the Second Test Series

The active immunization also shows us clearly that in spite of the low number of animals tested the complement plays its part in the creative process of building up rabbit immunity against carbunculosis.

Conclusions

The outcome of our experimental research work is -- speaking without any intention of generalizing our observations -- that complement inactivation of the blood leads to a reduced organic resistance, at least as far as the rabbit is concerned. This is why even in cases of active immunization of an animals or of seroprophylaxis applied to it the subjects died of carbunculosis, so that there must be a considerable importance attached to the complement in the build-up of an artificially created immunity of a rabbit against such an infection.

Furthermore, our experiments also led to another observation of certain interest;

For a long time the inhibitive action of the arsenobenzols on the development of hematic carbunculosis has been a well known matter in laboratory work as well as in research on living subjects and the above-mentioned products have been employed in the therapy of carbuncular infections. Based on the findings of Vallee and others we know that the arsenobenzols probably protect a certain number of rabbits against carbuncular septicemia if the drug is inoculated 12 hours after the germ.

In human medicine the use of neosalvarsan has been recommended with a dosage of 0.45 to 0.60 gms per day. As a matter of fact we never obtained therapeutic action in rabbits against carbunculosis with "Neojacol"; whenever we inoculated the arsenic compound we had to register the death of the animals right after the injection of poisonous germs.

On the other hand it must be noted that we saturated the organism of the rabbit by means of inoculations of up to

0.20 gms per kg of body weight, which corresponds to half the doses generally used in human medicine. Based on the above comments we dare say that the alleged lack of parallelism between anti-carbuncular immunity and bacteriolysins (maintained by various authors) may be due to the fact that for research work in the development of such antibodies people have mostly been under conditions that are far different from the conditions of the living organism without realizing that blood serum mainly used for laboratory work is sometimes very different from circulating blood.

Summary

The tests carried out by the author have brought us to the conclusion that in a rabbit immunized against hematic carbunculosis the inactivation of blood complement would lead to such a reduced organic resistance as to eventually cause the death of the animal.

Camerino, 23 July 1947.

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